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Characterization of Maze Performance in Adrenalectomized Sleep Disrupted Rats: A Comparison of Radial Arm Maze Performance between Adrenalectomized and Sham Adrenalectomized Sleep Disrupted Rats

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14. ABSTRACT
Sleep disruption affects performance in rats in several behavioral paradigms. Elevation of plasma corticosterine is associated with stress, and with sleep disruption in rats. Elevated corticosterone may contribute to dendritic regression in the hippocampus, a brain region associated with spatial learning. The working hypothesis of this study was removing adrenal glands in a rat will ameliorate the effects of sleep disruption on maze performance. Animals adrenalectomized and sham adrenalectomized were trained to criterion in the eight arm radial maze, and sleep disrupted for 12 hours during the light phase using a modified flowerpot in a cage with an inch of water. Removal of adrenal glands improved post sleep disruption performance slightly, but not significantly. However, the Barnes maze results showed an improvement of performance with a glucocorticoid receptor antagonist given 4 hours before the conclusion of 12 hours of sleep disruption on the flowerpot the post sleep disruption performances were compared. In addition, animals were implanted with a venous jugular catheter and sampled over a 36 hour period which included 12 hours of recovery. The results showed a significant elevation of corticosterone during the period of sleep disruption with recovery to cage control levels within 4 hours after return to home cage. In conclusion the sum of the performance impairments associated with sleep disruption may be the result of elevated corticosterone.

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Abstract:

Sleep disruption is stressful. Sleep disruption affects performance in rats in several behavioral paradigms. Elevation of plasma corticosterone is associated with stress, and with sleep disruption in rats. Elevated corticosterone may contribute to dendritic regression in the hippocampus, a brain region associated with spatial learning. The working hypothesis of this study was that removing adrenal glands in a rat will ameliorate the effects of sleep disruption on maze performance. Animals adrenalcetomized and sham adrenalectomized were trained to criterion in the eight arm radial maze, and sleep disrupted for 12 hours during the light phase using a modified flowerpot in a cage with an inch of water. Removal of adrenal glands improved post sleep disruption performance slightly, but not significantly. However, the Barnes maze results did show an improvement of performance with a glucocorticoid receptor antagonist given 4 hours before the conclusion of 12 hours of sleep disruption on the flowerpot the post sleep disruption performances were compared. In addition, animals were implanted with a venous jugular catheter, and sampled over a 36 hour period which included 12 hours of baseline, 12 hours of sleep disruption during the light phase and 12 hours of recovery. The results showed a significant elevation of corticosterone during the period of sleep disruption with recovery to cage control levels within 4 hours after return to home cage. In conclusion, some of the performance impairments associated with sleep disruption may be the result of elevated corticosterone.

Summary:

All three aims of assessing the effects of sleep disruption on radial arm maze, Barnes maze performance, and measuring plasma corticosterone have been completed. The physiological data of long term potentiation was difficult to collect because the animals were so fatigued by the experiment, and succumbed to anesthesia. The groups of animals on a large platform for sleep disruption were eliminated because there were not significant differences between the large and small platform in behavioral data. The radial arm maze data did not have significant differences between groups, however there was a trend for sleep disrupted animals to make more errors. The use of adrenalectomized animals in the radial arm maze study introduced some confounds because the animals appeared weak compared to sham adrenalectomized. In addition, the flowerpot method of sleep disruption was modified to compensate the apparent compromised physical condition of the adrenalectomized animals. The Barnes maze results support the hypothesis that blocking glucocorticoid receptors helps to improve performance impairments of sleep disruption. Corticosterone levels were significantly greater than cage control 3 hours in the sleep disrupted animals, as expected. Elevated corticosterone was associated with the performance impairments of sleep disruption.

Introduction

Fatigue contributes to increased aviation accidents. The interaction between fatigue and cognitive impairments is of vital interest. A better understanding of the neural mechanisms of sleep disruption will lead to more effective ways to minimize cognitive impairment when fatigue is unavoidable. This study utilized an animal model for REM sleep disruption to investigate the effects of fatigue on performance of a learned task. Two mazes which require hippocampal processing of spatial memory were used. The overall goal of this study was to investigate the effects of corticosterone on the impairments of sleep disruption. To achieve the goal of the study, the effects of corticosterone on the impairments of sleep disruption behavior; maze performance and physiology, long term potentiation (LTP) in the hippocampus were evaluated.

Characterization of the stress response unique to sleep disruption has been difficult. The word *stress* is ambiguous. Stress involves an increased activation of the hypothalamus-pituitary –adrenal axis. The stress response of sleep deprivation differs from other stressors because the levels of ACTH are not as high and the levels of corticosterone increase slowly and gradually. In addition, there is no habituation to the effects of sleep deprivation, with other stressors repeated exposure diminishes the HPA axis response (Meerlo, Koehl et al. 2002). The behavioral and physiological response of that increased hormonal activity is of interest in examining the effects of sleep disruption. Sleep disruption increases plasma corticosterone. One measurement of stress in rats is plasma corticosterone. Stress in animals has been associated with decrease in LTP in the hippocampus (Yamada, McEwen et al. 2003). Restraint stress has been shown to impair LTP in rats (Foy, Stanton et al. 1987). Rats exposed to cats had elevated corticosterone, but not LTP impairments, primed burst potentiation were impaired (Mesches, et al., 1999). LTP may be induced with high frequency tetanus stimulation, a more physiological relevant stimulus given at theta rhythm still revealed impairment of LTP in the dentate gyrus after stress. Neural mechanisms underlying the effects of stress on LTP are undefined. Corticosterone has been considered a possible modulator of the effects.

Corticosterone mediates its effects through the glucocorticoid receptors (GR). Hippocampal neurons express a high density of these receptors. The glucocorticoid receptor is a transcription factor that is translocated to the nucleus and binds to specific response elements. GR only bind in high concentrations of corticosterone, which diurnally increase during the dark phase. In rats, GR element binding in the hippocampus is greater than other brain regions when the plasma corticosterone levels are equivalent. This finding suggests that there is regulation of intracellular response to corticosterone by GR binding (Kitchener, Di Blasi et al. 2004). Adrenalectomy (ADX) also changes the binding of steroid receptors. ADX increase GR binding capacity (O'Donnell, Francis et al. 1995). Corticosterone modulates hippocampal synaptic changes, as shown by the significantly increase in spine density of CA1 pyramidal neurons. induced by

dexamethasone a glucocorticoid receptor agonist (Komatsuzaki, Murakami et al. 2005). This study used ADX to study the effects of elimination corticosterone. However, adrenalectomy has numerous other effects on rat brain and behavior.

More specifically, adrenalectomies have several reported effects on the hippocampus. Anatomical effects on the hippocampus are multiple. There is marked granule cell loss (Gould, Woolley et al. 1990). The loss of granule cells at four months post adrenalectomy may be up to 43% (Sousa, Madeira et al. 1997). In addition to cell loss, cognitive impairments have been noted after adrenalectomies in rats (Sloviter, Valiquette et al. 1989; Armstrong, McIntyre et al. 1993; Islam, Henriksson et al. 1995). In one study spatial learning of the water maze was impaired at 12 weeks post adrenalectomy and not at 22 weeks post adrenalectomy. Replacement of corticosterones after adrenalectomy did reverse learning impairments in the water maze, suggesting the impairment is due to neuronal loss and not the acute effects of loss of corticosterone (Conrad and Roy 1993). The neuronal loss may be attributed to several effects of corticosterone. The mechanism of cell death appears to involve an interaction between corticosterone and expression of the pro apoptotic gene bax in granule cells of the dentate gyrus. Subcutaneous administration of corticosterone prevented the increase of bax after adrenalectomies (Cardenas, Parra et al. 2002). It is reasonable to expect adrenalcetomies to increase apoptosis in the dentate (Greiner, Cardenas et al. 2001). In addition, CA3 pyramidal cells are vulnerable to high levels of corticosterone. The inconsistencies of effects support the use of agonist and antagonist in adrenalectomized and sham operated animals to gain more insights in to the interactions of stress and cognitive impairment.

Adrenalectomies' effect on neurotransmitters systems includes changes in neurotrophins, and cholinergic receptors. The expression of neurotrophin NT-3 mRNA in the hippocampus has been shown to be reduced compared to sham operated rats (Grundy, Patel et al. 2004). Further evidence for effects of corticosterone on neurotrophins is that BDNF mRNA is increased in the hippocampus after an adrenalectomy and brain trauma. Corticosterone replacement blocks this response to brain trauma (Grundy, Patel et al. 2000). Decreases in plasma corticosterone alter spatial learning in rats. Corticosterone modulates muscarinic acetylcholine receptors through the mineralosteoroid receptor. Changes in muscarinic receptors may alter the state of the hippocampus during memory and learning (Douma, Jansen et al. 1999). In addition, the relationship between serotonin and corticosterone suggests that corticosteroids regulate $5HT_{1A}$ receptors (Chennaoui, Drogou et al. 2003). Sleep deprivation increases serotonin in the hippocampus (Youngblood, Zhou et al. 1997).

There are several methods to block the effects of corticosterone. One is to remove the adrenals and another is to block the receptors for the hormones with a glucocorticoid receptor antagonist. Blocking the actions of elevated

corticosterone associated with sleep disruption helps to distinguish the effects of sleep disruption from a general stress response mediated through corticosterone. A refined perspective on sleep disruption stress could reveal insights for mitigating the stress of sleep disruption to improve the quality of sleep so that less sleep gives more recovery of performance.

Sleep deprivation impairs learning in spatial tasks in rats. Sleep deprivation preceding training in the Morris water maze leads to poor performance (Guan, Peng et al. 2004). Sleep deprivation during the four hours after daily training session in the Morris water maze also causes poor performance (Smith 1996; Smith and Rose 1997). Sleep deprivation before training in animals adrenalectomized and given corticosterone pellets to replace corticosterone and eliminate the elevation of corticosterone in response to sleep deprivation required more trials to be trained in the Morris water maze compared to intact cage controls. One conclusion was that rapid acquisition of spatial task requires a normally functioning hippocampus and not a hippocampus affected by sleep disruption (Ruskin, Dunn et al. 2006). Sleep disruption has been shown to alter the strategy used by rats to find the target box in the Barnes maze (Mendez-Diaz, Irwin et al. 2005). The radial arm maze and the Barnes maze are considered to be solved by the animal's use of a spatial map. The radial arm maze requires several days of food restriction prior to and during training. The food restriction may introduce a confound in assessing the effects of sleep disruption because food restriction is associated with an increase in orexin, which is also increased by sleep disruption. The Barnes maze does not require food restriction, because the animal is inclined to find a dark box to escape the open brightly illuminated maze surface. This study used two different mazes to better characterize the effects of sleep disruption on performance.

Purpose: The purpose of this study is to investigate the presumed role of corticosterone in the stress response to sleep disruption. In addition, this study will utilize two behavioral tests, the radial arm maze and the Barnes maze and physiological measure of hippocampus state, LTP, to examine the relationships between stresses, sleep disruption and performance impairments.

The working hypothesis is that blocking of corticosterone effects will lessen the impairments in maze performance associated with sleep disruption.

Methods:

Behavioral Tests:

1. Eight arm radial maze

Animals: Male Sprague-Dawley rats weighing 175-199 g.

1 Eight arm radial maze manufactured by Lafayette Instrument Company. The maze is 72 inches in diameter and 36 inches above the floor and constructed of

black painted stainless steel with Plexiglas walls on the arms and hub. Each arm is 10 inches across. The doors on the hub are controlled by levers behind curtains.

2. A laptop with HVS software to collect tracking data on the eight arm radial maze.

RAM as a tool to test reference memory. The radial arm maze was developed by Dave Olton to test the ability of a rat to remember a list of spatial locations. Several experiments demonstrated the rat discriminates between arms based on a map in its memory, not response strategies such as choosing alternating arms or intra-maze cues such as food or trail odors. Extra-maze spatial cues are used. As a tool, all eight arms baited tests for working memory or a memory of which arm has been visited in a given trial. In this study all eight arms are baited with one half a Froot loops. In addition to each of four walls having a paper cue consisting of a large black and white pattern approximately 3 feet by 2 1/2/ feet, an additional line drawing on a clear film was affixed to the Plexiglas at the end of each arm. In addition, animals were given two trials a day, one in the morning and one in the afternoon between 3 pm and 6 pm. Animals were trained to criterion, which are eight correct choices out of the first eight choices. On the morning following an animal's criterion trial the animal was either left in the home cage during the light phase, 7 am to 7 pm, or placed on an inverted flowerpot to induce REM sleep disruption. The inverted flowerpot created a circular platform approximately 2.3 inches in diameter. The flowerpot was inverted in a Plexiglas cage filled with 1 inch of tap water. The animal may walk in the water, but will not sleep. The platform allows for slow wave sleep, but not REM sleep because REM sleep causes loss of muscle tone and the animal would fall into the 1 inch pool of water.

At the end of the light phase, 7 pm, the animal was given a final maze trial. The expected outcome for a cage control is to have a very small error number. In pilot studies sleep disrupted animals made significantly more errors on the post sleep disruption trial.

Initially, animals were anesthetized with urethane to measure long term potentiation immediately after the maze trial. However, these animals were succumbing to anesthesia, and so animals were permitted 12 hours of recovery before attempting to anesthetize for physiological measurements.

Protocol for 8 arms baited:

Maze training was:

1. Day 1, 10 minutes with all doors to arms open, exploration. No bait.

2. Day 2, the arms have ½ a Froot loop, and the animal is allowed up to ten minutes to visit each arm. The trial is stopped after 10 minutes or when the animal has visited each arm at least once.
3. Day 3, two trials a day consists of baiting all the arms, and watching for the animal to visit each arm, the trial is stopped once the animal has gone to the bait and returned to the hub of the maze for each arm. The time is called the duration. Mistakes are counted when an animal revisits an arm by going at least 1/3 of the length of the arm.
4. The animal reaches criterion by visiting each arm just once.
5. Once the animal reached criterion, the animal was placed in one of two groups for sleep disruption the next day.
6. One group of animals was placed on a small platform to induce REM sleep deprivation, and the other group remained in the home cage. The time spent on the platform was 12 hours, during the light phase.
7. The animals had a maze trial immediately after the 12 hours of sleep disruption or in the case of cage controls after 12 hours in the home cage.

2. Barnes Maze:

The Barnes maze was built at UTSA and was a circular tabletop 48 inches in diameter with 20 holes 4 cm in diameter cut along the perimeter. This behavioral test does not require food restriction, as does the radial arm maze. The Barnes maze should provide another behavioral test that detects hippocampal impairments eliminating of food restriction. Food restriction increases corticosterone and orexin, which are increased by sleep disruption. Based on the literature and preliminary trials the protocol is as follows:

1. Animals will be given four trials a day with a 10 minute rest period between trials.
2. All the surfaces of false boxes and the target box will be wiped with a Sani cloth plus between each trial.
3. The first day will be habituation to the Barnes Maze.
4. If the animal fails to discover the target box within the three minutes allotted for each trial, the animal will be placed into the box, for one minute.
5. Once in the target box the animal remains in the target box for 1 minute during all phases of training and testing.
6. On the day following habituation, animals will be placed in a cylindrical Plexiglas start tube in an orientation random to the target box for 30 seconds.
7. The start tube will be raised by hand. Rats will be allowed to explore the maze until the animal enters the target box or 3 minutes has elapsed.

8. Rats will return to home cage between trials for a 10 minute interval.
9. Latency to entering the target box was recorded in seconds. Number of errors defined as sniffing over or poking around an incorrect hole, was also recorded.

The room of the Barnes maze is relatively small; all four walls have differing black and white patterned cues. The Barnes maze on the first trial of each day tests reference memory, the subsequent 3 trials test a combination of working and reference memory.

Trials were administered for four days; animals were then assigned to one of three groups. One group was sleep disrupted for 12 hours during the light phase with no injection. The second group was sleep disrupted for 12 hours during the light phase and injected with RU 486 sub cutaneously approximately 3 hours prior to the final set of trials in the Barnes maze. The 100 mg of RU 486 was diluted in the bottle by adding 10 ml of Propylene glycol (Sigma Propylene Glycol 200). The dilution was 0.1 g/10 ml, so the dose will be 0.4 ml per 200 gram animal or 20 mg/kg. Animals injected with the vehicle of propylene glycol were given 0.4 ml.

The third group remained in the home cage, and received a sub cutaneous injection of propylene glycol approximately three hours prior to the final maze trial. Animals were tested for four trials as was done during training in sets of two, and given the ten minute rest between each of the four final trials.

Surgical Protocols

1. Venous jugular catheterization:

Following induction an adequate level of anesthesia with IP solution the subject was clipped closely in the scapular, right neck to jaw, clavicular and upper thoracic areas. The inter scapular midline was marked on the back side. The clipped areas and right front leg and paw were swabbed with beta dyne solution. The subject was placed supine on the sterile drape operative platform which maintains a slight head-down position. The forepaws were restrained laterally and slightly cephalic. A sterile drape was fenestrated and positioned exposing the right neck and clavicle.

The subcutaneous external jugular vein was much larger than the deeper internal jugular vein in the rat, and is a better choice for catheterization. A vertical incision on the skin was placed midway between the midpoint of the sternum and point of the shoulder. The incision extended approximately 0.5 cm below the clavicle to 1.5 cm above. The incision was carried through the skin, and subcunteous tissue with care to visualize the vessels underneath. Lateral traction during dissection was helpful. Cotton swabs were used in opposition and

were effective in separating most of the subcuticular fine soft tissues superficial to the external jugular vein. The vein was exposed where it passed deep to the muscle on the chest. Cotton swab dissection cleared the anterior surface of the vein cephalad for approximately 1 cm. Branches entering the jugular laterally from the shoulder area were noted. Any bleeding encountered was easily controlled with pressure from swabs.

Two lengths of about 4 cm from the silk-suture end were snipped. With fine 45 degree angle tissue forceps, the level where the vein passes beneath the pectoral muscle was carefully dissected by inserting the forceps tips and allowing them to widen, gradually creating a tunnel beneath the vein. A length of silk was passed behind the vein at this point carefully to avoid twisting the vessel. The vein was not dissected out of its bed along its length. Using the fine forceps technique, aided by the swabs the jugular was encircled above (cephalic to) the branch. The remaining silk tail (do not remove the needle) was used to ligate the jugular, leaving the knot tails attached. Control of all branches prevented back-bleeding during catheter insertion. Lifting on the untied silk at the muscle level controlled any bleeding.

The catheter was prepared by flushing with sterile saline. With smooth 45-degree forceps, gently the vein was grasped (just below the lateral branch) and closed by pinching. The forceps were held perpendicular to the neck to pinch. Lay the forceps medially, rotating the vein so it can be partially viewed from the side at the pinch. Incise the venotomy about one third across the vein by placing the tip of a sharp #11 scalpel (with blade up) and pushing it smoothly across the pinched vessel near the steadying forceps. Any back-bleeding was controlled by pulling up on the proximal silk. With the other pair of 45 degree forceps, the catheter was grasped at about 2 mm from the tip and advanced it into the other vessel toward the clavicle. (The vessel may be dilated by passing one of the forceps jaws within, if necessary.) The catheter has a PE spool for tying at approximately 18 mm from the tip of the silastic portion. The silastic tubing was advanced into the vein until the spool was at the venotomy. Venous blood return was checked by attaching the saline syringe to the 23 gauge leur tip adapter and aspirating the catheter. When return was confirmed, the catheter was flushed with saline and the silk pre-positioned suture was tied down at the clavicle-muscle juncture. Care was used to avoid compressing the catheter. Venous return must be confirmed frequently during the remaining procedure. Tie the spool to the vein using the silk tails which have ligated the distal jugular. All knot tails were trimmed after venous return was confirmed.

A subcutaneous tunnel catheter tunnel was created with curved clamps from the neck incision over the right shoulder toward the inter-scapular midline. Note carefully the direction of any curve in the catheter 'memory' to avoid twisting on passage through the tunnel.

2. LTP in vivo:

Glass micropipette recording electrodes (FHC o.d. 1.2mm, i.d. 0.6mm) were prepared and filled with 3 M NaCl. The stimulating electrode was tungsten (FHC Concentric bipolar). Coordinates of the recording electrode were approximately 4.5 mm posterior to bregma and 2.0 mm lateral to midline. Coordinates of the stimulating electrode were 8.0 mm posterior to bregma and 5.2 lateral to midline. The dentate gyrus recording site was identified by single unit activity characteristic of granule cells. LTP was induced with 50 μsec duration monophasic constant current pulses. The range of stimulation was 150-300 microamps. After 30 minutes of baseline four sets of titanic stimulation trains separated by 10 minute intervals at 30, 40, 50 and 60 minutes were administered to induce LTP. Each set contained 5 trains, 10 pulses per train 400 Hz delivered at a rate of 1 train per second for 5 seconds. The pulse widths in the trains were 50, 100, 150 and 200 μsecs. The pEPSP were recorded every minute until the experiment was terminated 70 minutes after the first tetanus had been applied. Placement in the dorso medial perforant path was verified with a 100 Hz titanic stimulation at the end of the experiment.

3. LTP in vitro:

Animals were anesthetized with pentobarbital (60 mg/ Kg) and euthanized by decapitation. The brain was rapidly removed to cold Kreb's Ringer (KR) solution saturated at 95% O2 and 5% CO2. It was immediately trimmed, and 400 μm slices were cut on a vibratome transverse to the long axis of the hippocampal formation. Slices were incubated in KR for two hrs at 33°C. An individual slice then was placed on a fenestrated Plexiglas plate in a submerged recording chamber which was perfused with oxygenated KR solution at 34°C at a flow rate of 3 ml/min. For morphological analysis, Lucifer yellow CH (Sigma, 10% in distilled water) was injected into a cell through a special recording electrode by application of 2 nA negative current pulses of 250 ms duration at 2 Hz for 2 min.

All protocols were performed in accordance with the UTSA IACUC protocol number RA 054-02/09A1, approved February 28, 2006.

RESULTS:

Radial Arm Maze

The radial arm had eight arms with Plexiglas wall and remotely controlled Plexiglas doors for each arm near the central hub. Animals were tested once a day and given ½ a Froot loop in all eight arms with all eight doors open simultaneously at the beginning of each trial. While the animals tested did not show a significant difference between groups, the trend was for adrenalectomized animals to make fewer errors on the post sleep disruption trials. The sham adrenalectomized animals made an average of 2.6 errors after

sleep disruption. The adrenalectomized animals made an average of 2.2 errors after sleep disruption despite their comprised physical state. The sham adrenalectomized cage controls made an average of 2.5 errors on the day following criterion. This average is higher than cage controls in an earlier study with an average of 0.5 errors in non operated animals using the same radial arm maze. A Kruskal-Wallis one way analysis of variance on ranks had an F value of 0.0499, and a p value of 0.956. The power is below the desired power, and might make it more difficult to detect difference between groups. In addition, the flower pot method was modified to one inch of water in a cage, not 12 inches in a deep barrel.

Barnes Maze

Animals sleep disrupted for 12 hours during the light phase using the modified flower pot method with a small platform in a pool of water 12 inches deep, did have an significantly increased latency to enter the target box compared to cage controls and sleep disrupted animals treated with a glucocorticoid antagonist, RU 486 on the first of four trials on the fifth day. The other 3 trials after sleep disruption did not show a significant difference. The number of nose pokes was not significantly different between groups on any trials, perhaps because the animals were seeking the target box as efficiently as possible on the first trial. The animals usually explored more on trials 2-4 during training after the location of the target box was learned and verified.

Corticosterone levels

The elevated levels of corticosterone during sleep disruption, are consistent with reports in the literature of a slow and steady increase of plasma corticosterone during the period of sleep disruption. The quick return to lower levels once sleep disruption is complete is of interest.

Anatomy

Dr Hori a visiting professor filled some cells from his studies on sleep disrupted animals. He contributed the images and is continuing the study to quantify the changes. The number of spines appears to increase with sleep disruption in the granule cells of the dentate gyrus.

In vitro LTP There was no significant difference between the groups of cage control and sleep disrupted animals. This study did not examine the effects of adrenalectomies, and was conducted on animals immediately after a twelve hour period of sleep disruption during the dark phase.

In vivo LTP in the sham adrenalectomized and adrenalectomized groups were all food restricted up to 3 weeks to complete the maze training, this may have

complicated the reliability of finding field potentials. Although each graph has an n=2, many other attempts were made, however the signals were not usable. This data was collected on animals within twelve hours of a twelve hour period of sleep disruption during the light phase.

Results of eight arms baited radial arm maze.

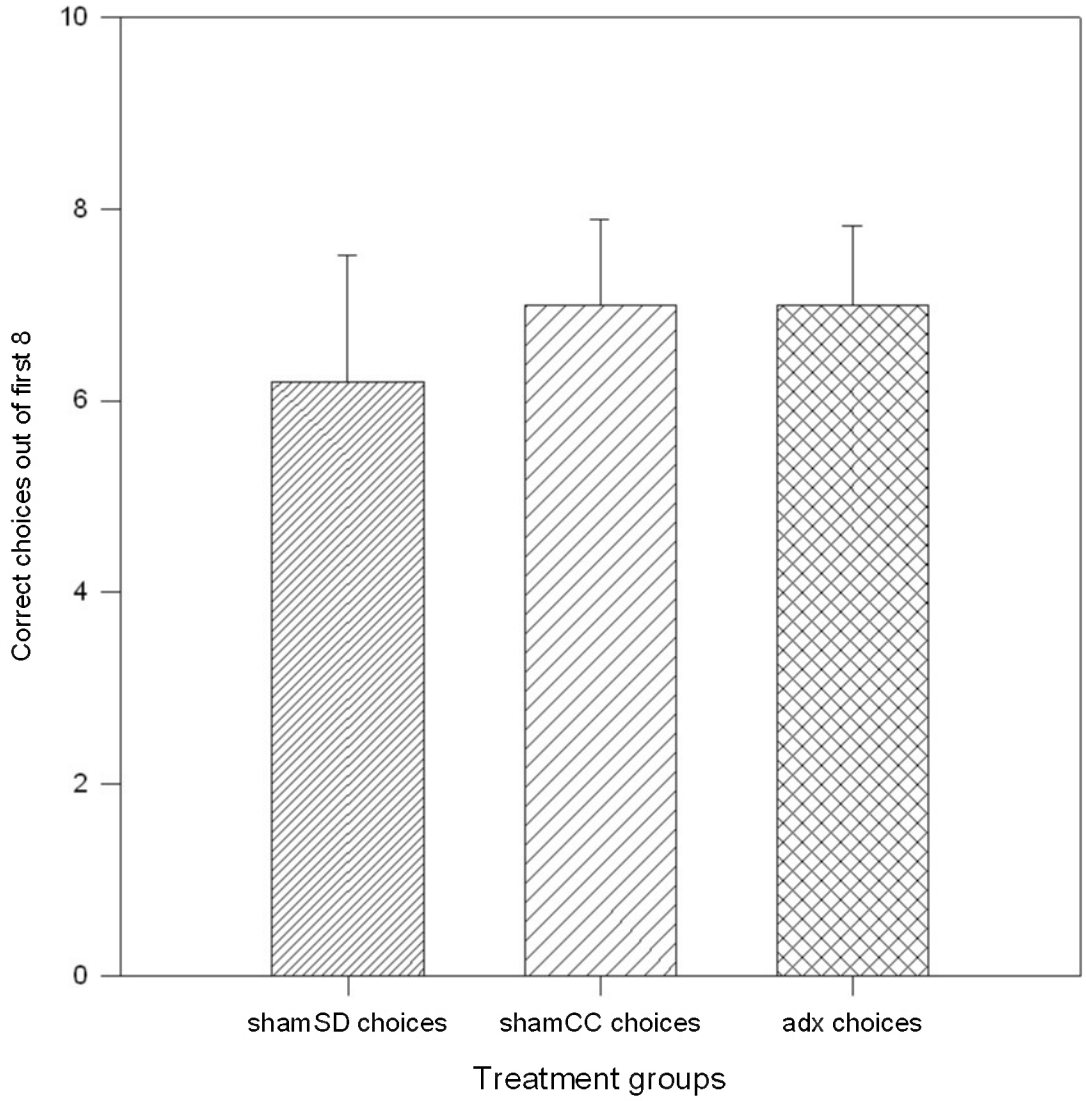


Figure 1. Comparisons of three groups correct choices out of first eight in the radial arm maze after 12 hours of sleep disruption. The sham *n=9,* sham cage control *n=5,* and adrenalectomized sleep disrupted *n=7*. There is no significant difference between groups. All animals were trained to criterion with two trials a day, one in the morning and one in the afternoon. All eight arms were baited with one half a Froot loop.

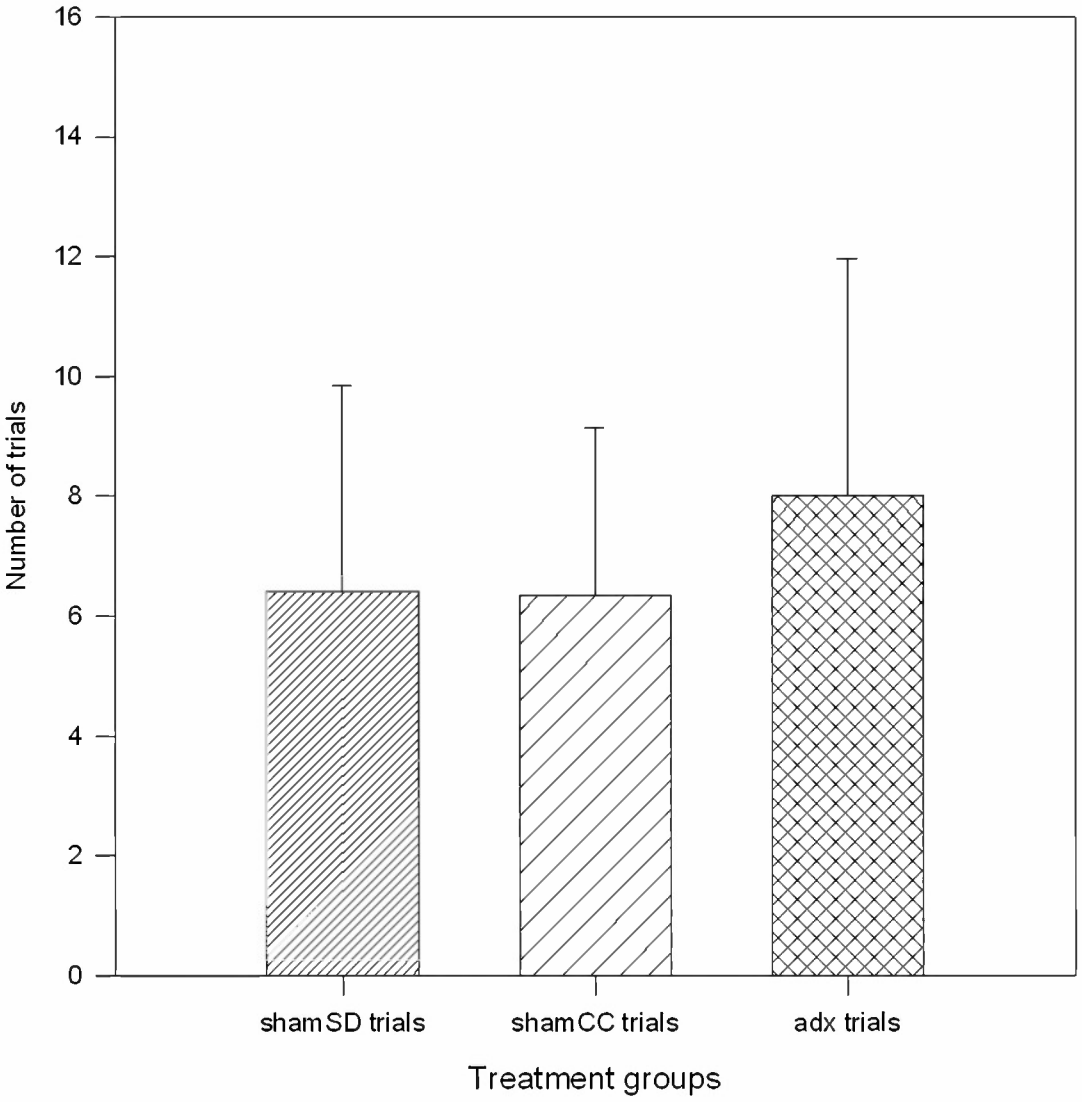


Figure 2. Comparison of number of trials to criterion in the radial arm maze. The mean number of trials to criterion was not significantly different between groups, which is as expected. Adrenalectomy did not significantly affect the acquisition curve.

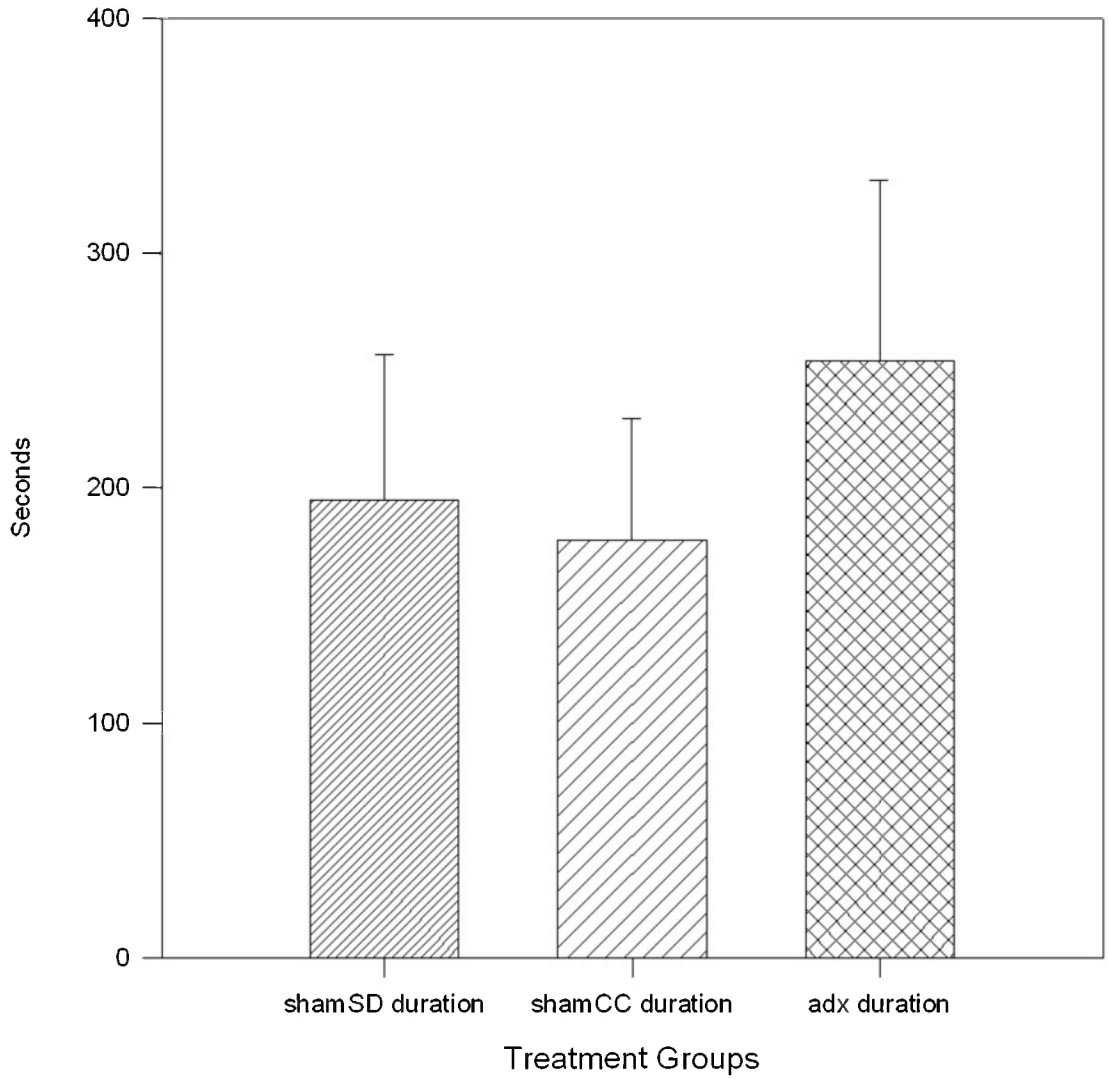


Figure 3. The comparison of the duration of the final trial in the radial arm maze. The groups did not have significant differences, although the adrenalcectomized animals tended to take longer to complete the final trial. This increase in duration may be due to increased fatigue, since the animals appeared more fatigued.

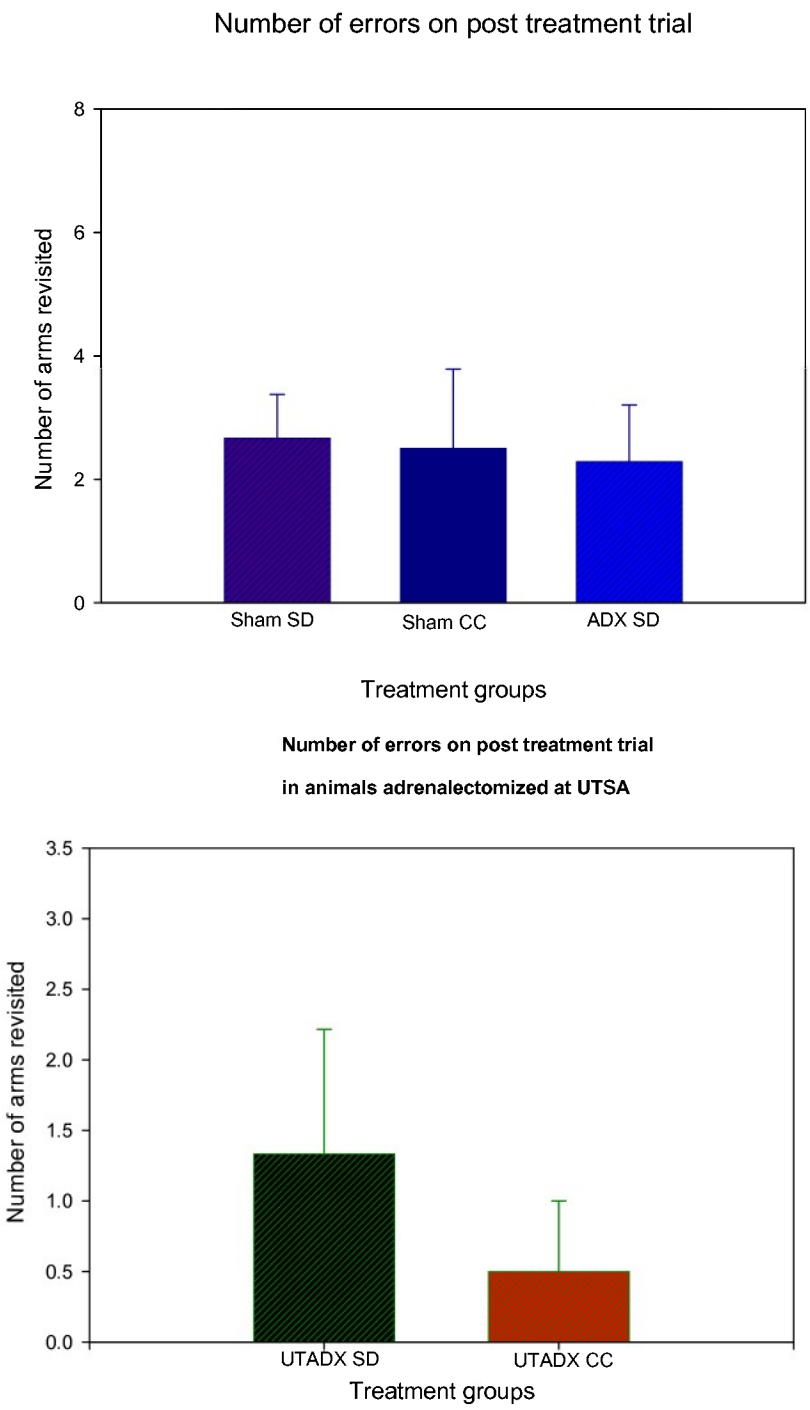


Figure 4. Comparisons between adrenalectomized and sham adrenalectomized radial arm maze performance in post sleep disruption trial. A. The sham group *n=9,* sham cage control group *n=6,* and adrenalectomized sleep disrupted (ADX SD) group *n=7.* There is no significant difference between groups. However, there is a trend for the adrenalectomized animals on average to make fewer errors. B. Comparison of sleep disrupted and cage control performance of male rats adrenalcetomized in a UTSA lab. Sleep disrupted group had n=3 and cage controls had n=3. These animals did not appear as fatigues by the surgery, and the differences between cage controls and sleep disrupted were consistent with previous radial arm maze studies. Abbreviations are SD –sleep disrupted, CC-cage control, not sleep disrupted, and sham –sham ADX.

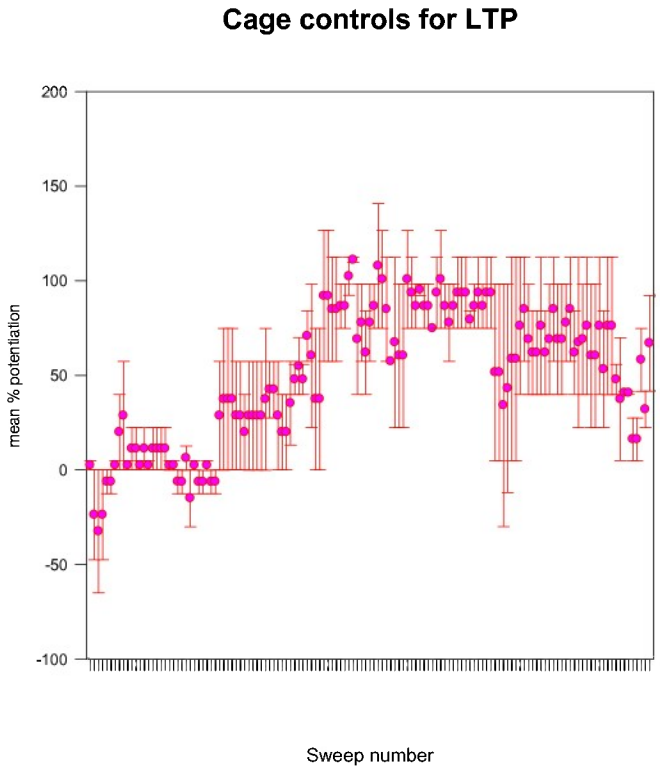


Figure 5. The mean per cent potentiation of cage controls. The animals did have potentiation averaging 50 in the final ten minutes. The tetanus stimulation appeared to have frequency facilitation. N=2. These controls are age matched. Error bars are standard error of the mean.

Mean per cent potentiation on sleep disrupted adrenalectomized rats

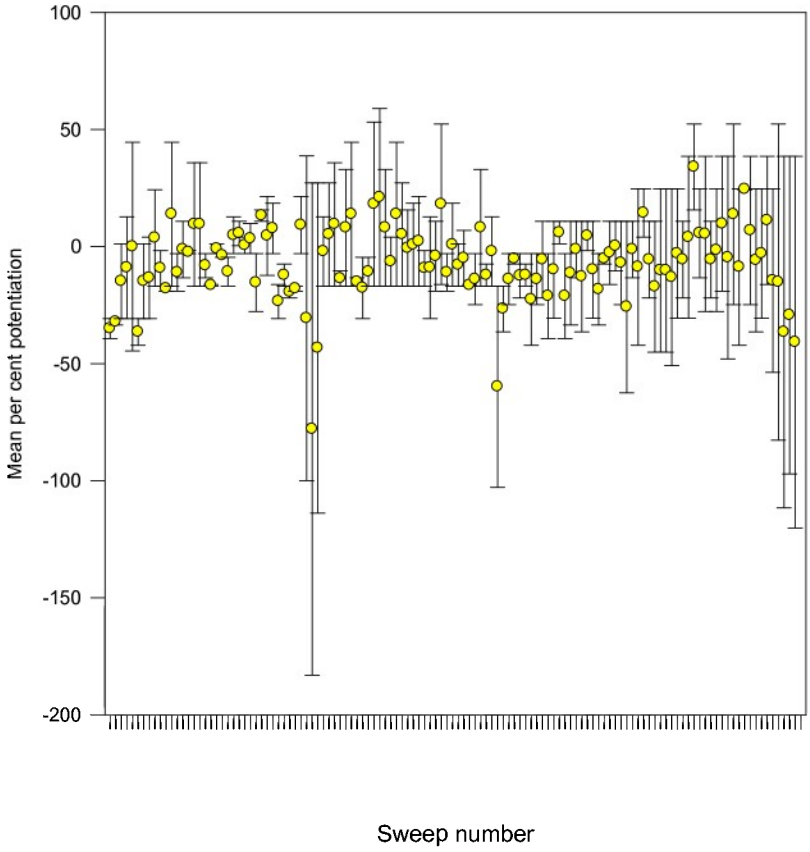


Figure 6. Mean per cent potentiation of adrenalectomized sleep disrupted animals. There is no potentiation. The animals were very fatigued. N=2. Error bars are standard error of the mean.

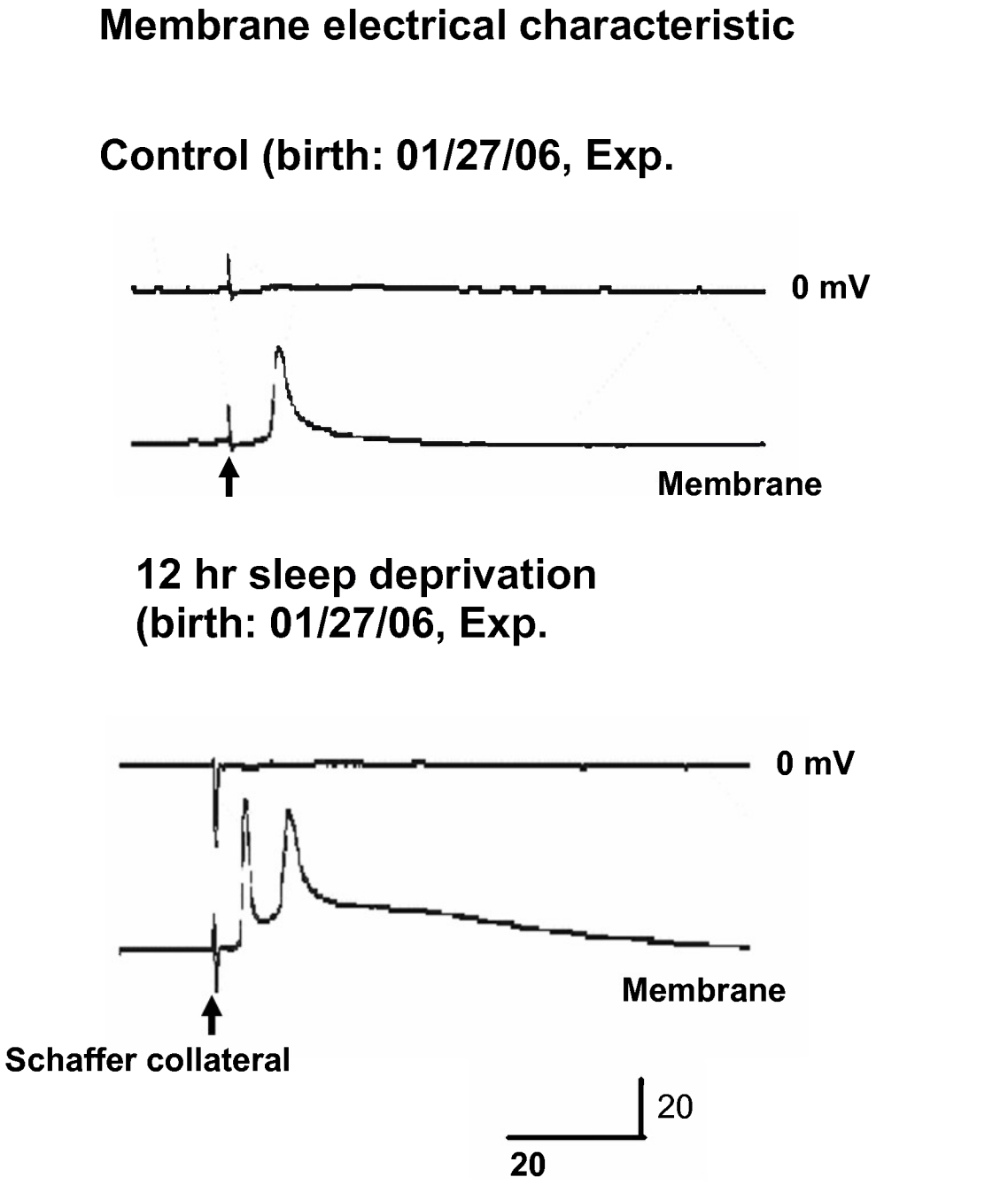


Figure 7. Membrane properties of neurons from sleep disrupted and non sleep disrupted animals. The sleep disrupted animal has two excitatory post synaptic potentials in response to the same stimulus that caused only one excitatory post synaptic potential in a slice preparation from a non sleep disrupted animal.

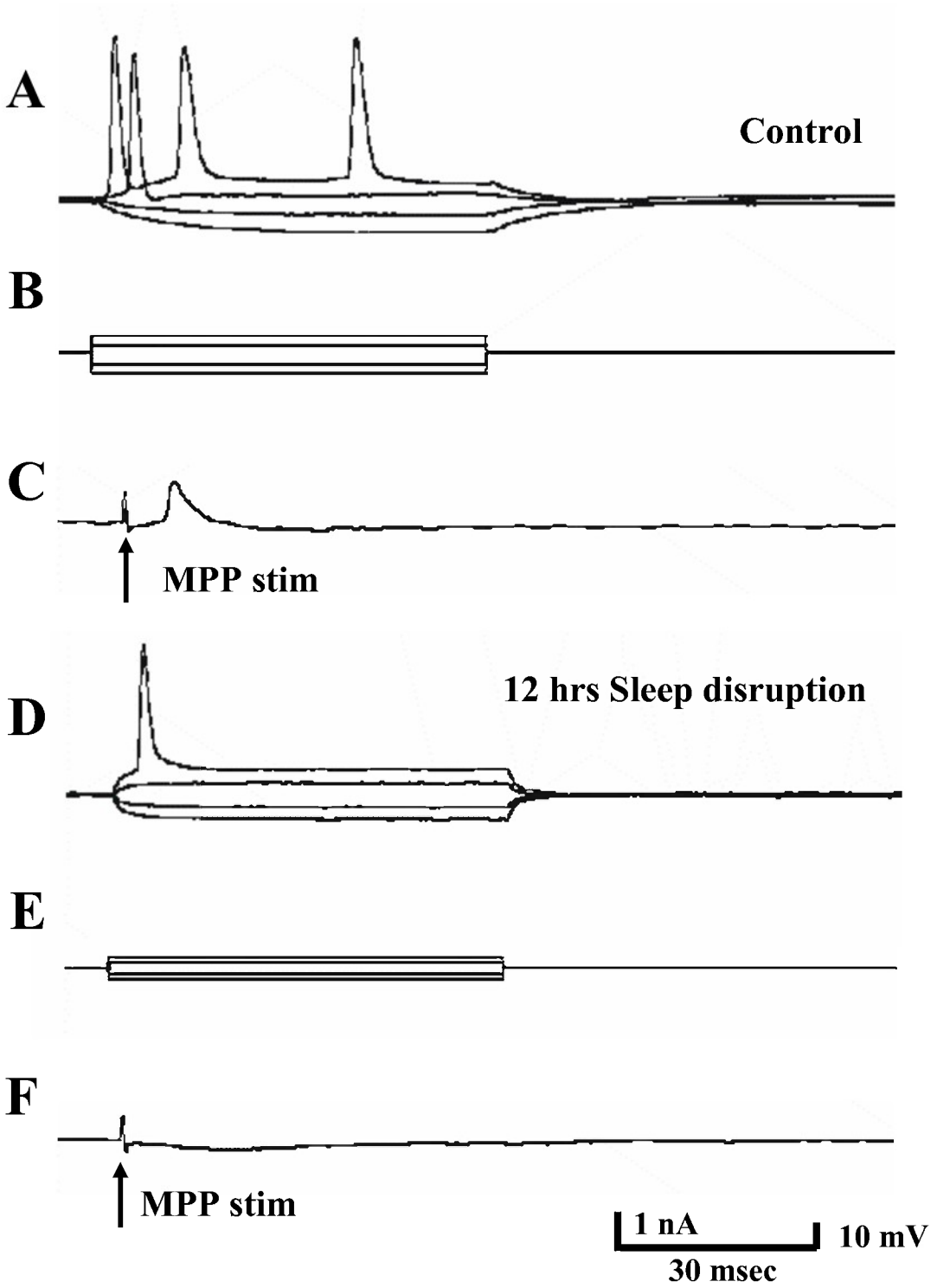


Figure 8. In vitro membrane properties of cells. Stimulation in the medial perforant path, (MPP) elicits a series of action potentials in a granule cell of a cage control. The response to the same stimulus in the granule cell of a sleep disrupted animal is much smaller in amplitude and frequency. These animals were male Sprague Dawley rats weight 200-250 g. A. Responses to intracellular pulses for control. B. Responses to a graded current. C. EPSP responses to a medial perforant path stimulation. D. Responses to intracellular pulses in a slice preparation from a sleep disrupted rat (290 g). E. Responses to a graded current in a sleep disrupted rat. F. Inhibitory post synaptic potential response to a medial perforant path stimulation in a slice preparation from a sleep-disrupted rat.

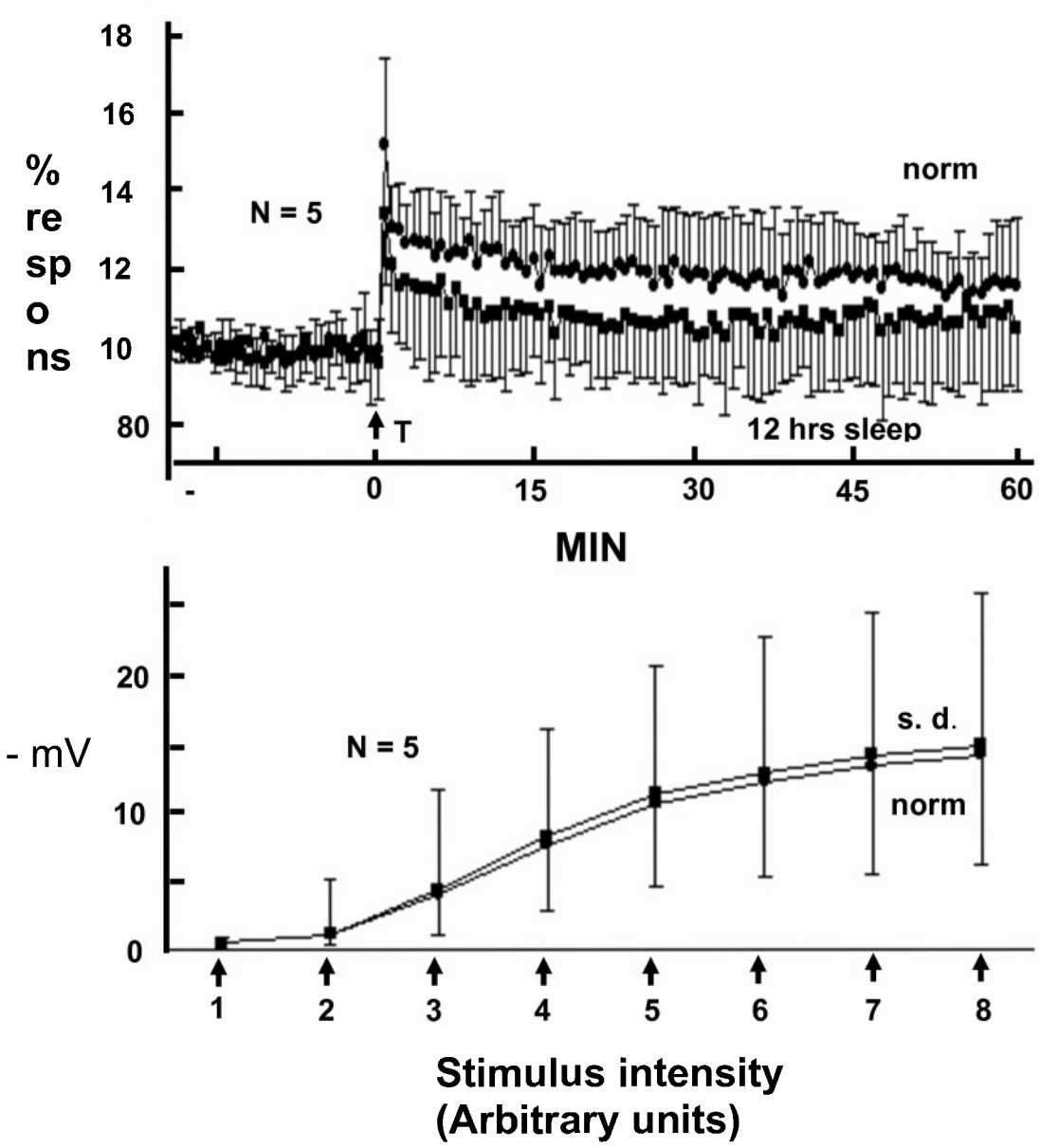
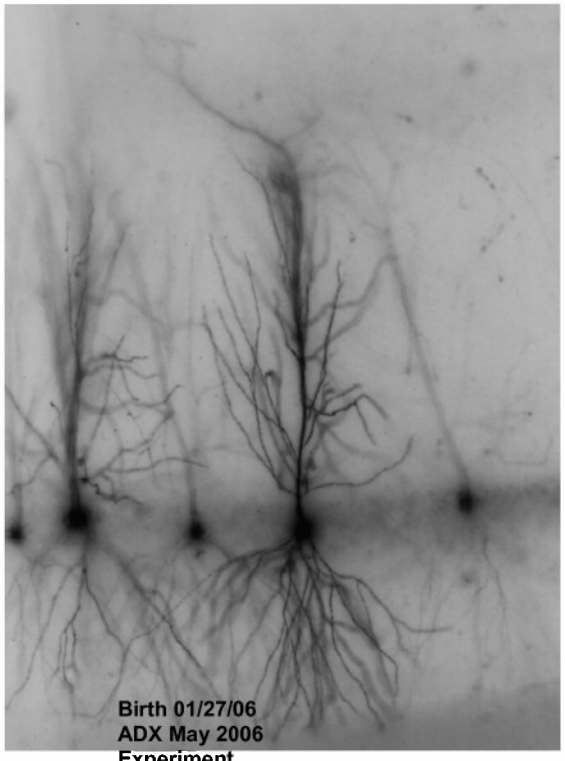


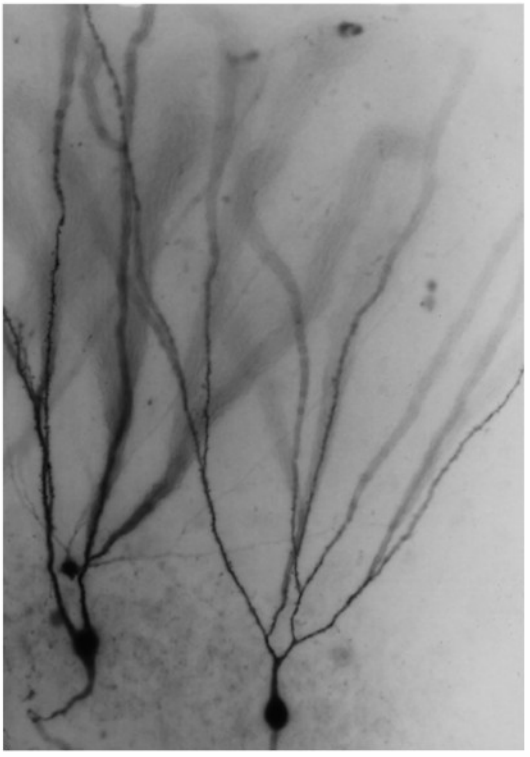
Figure 9. *In vitro* recording in the granule cells in which LTP is measured by the minimum slope over the upswing of the sweep and averaged per treatment group for each time point. The points are averaged from five slices from at least three animals. Sleep disrupted responses were impaired but not significantly less than cage controls.

Adrenalectomized rat control

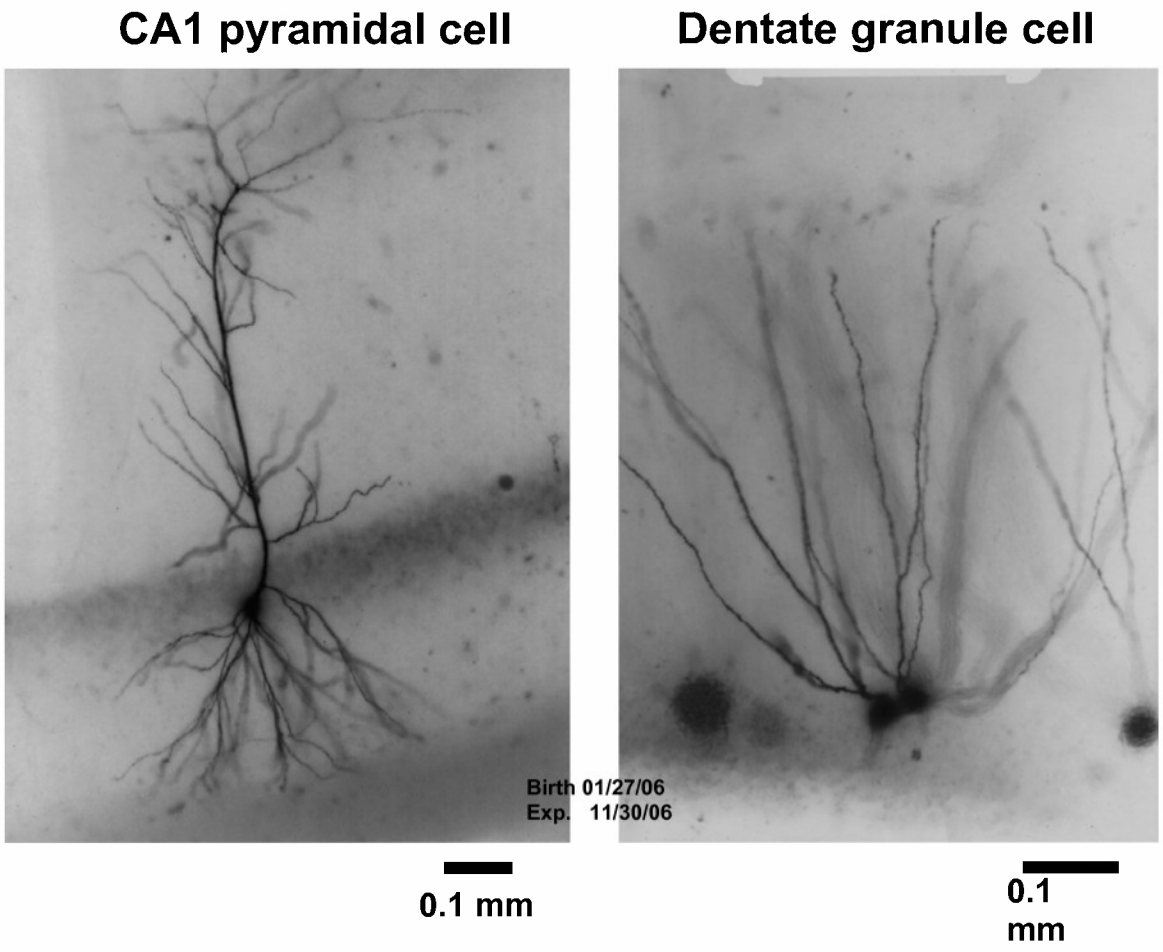
CA1 pyramidal



Dentate granule



Adrenalectomized 12 hr sleep deprivation



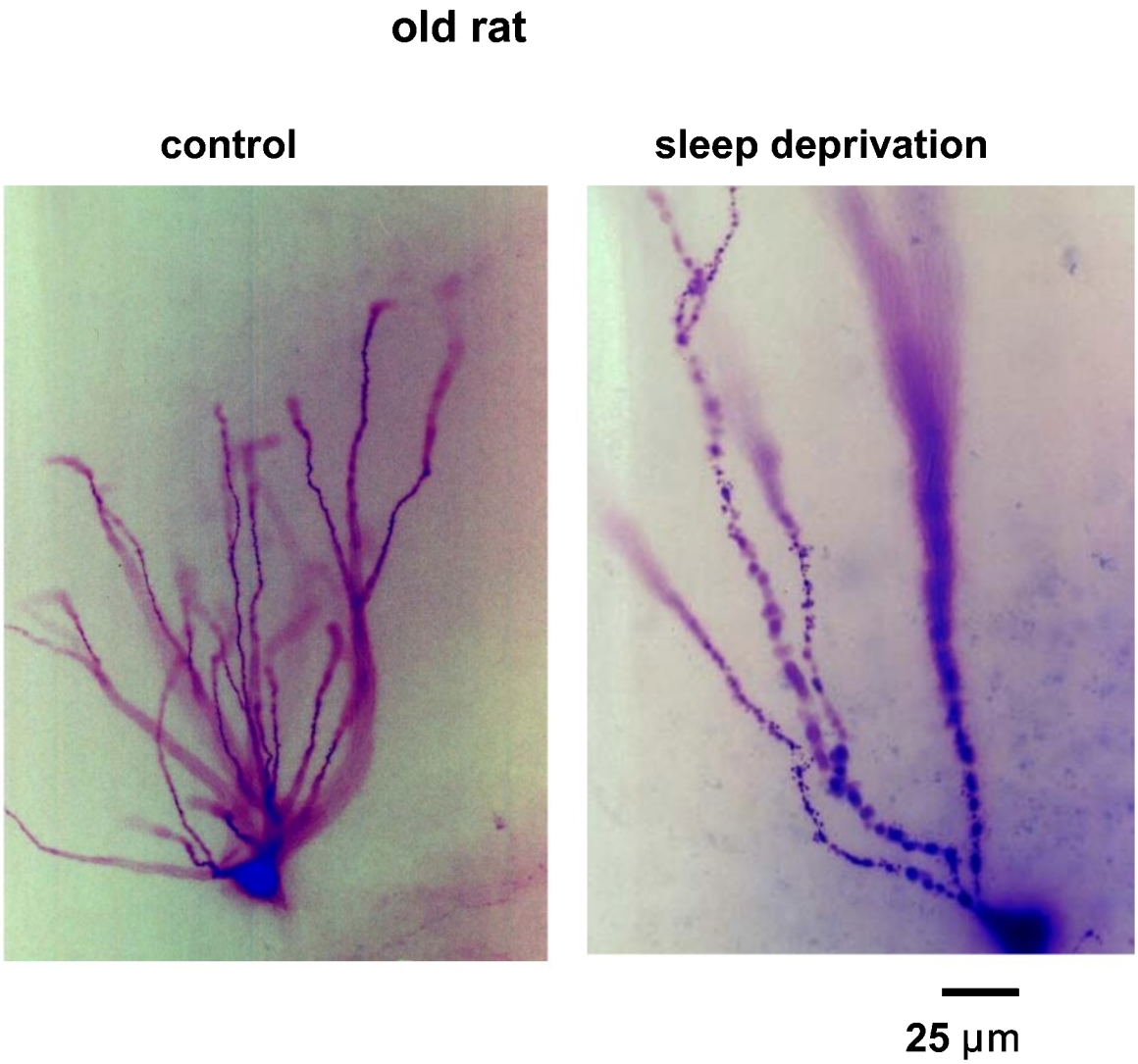


Figure 10. Images of filled neurons of the hippocampus from control and sleep disrupted rats. The granule cells from adrenalectomized sleep disrupted rats do not appear to have as many spines as the granule cells from sleep disrupted rats. These anatomical data suggests the loss of corticosterone protects against spine proliferation in response to sleep disruption. The photomicrographs were taken on a fluorescent scope by Dr. Hori, a visiting professor at UTSA.

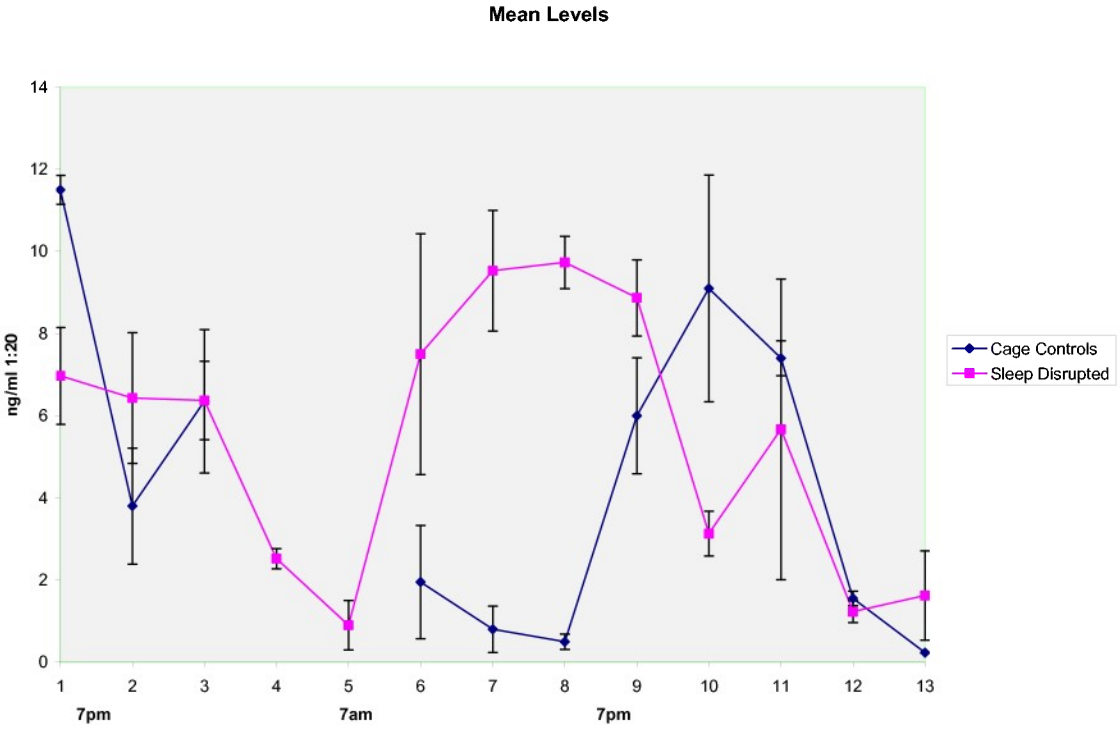


Figure 11. Preliminary results of corticosterone levels measured in rats. The profile of corticosterone levels over the 36 hours samples were taken appears to differ with sleep disruption. Corticosterone is elevated during the dark phase during sleep disruption, and drops during the light phase.

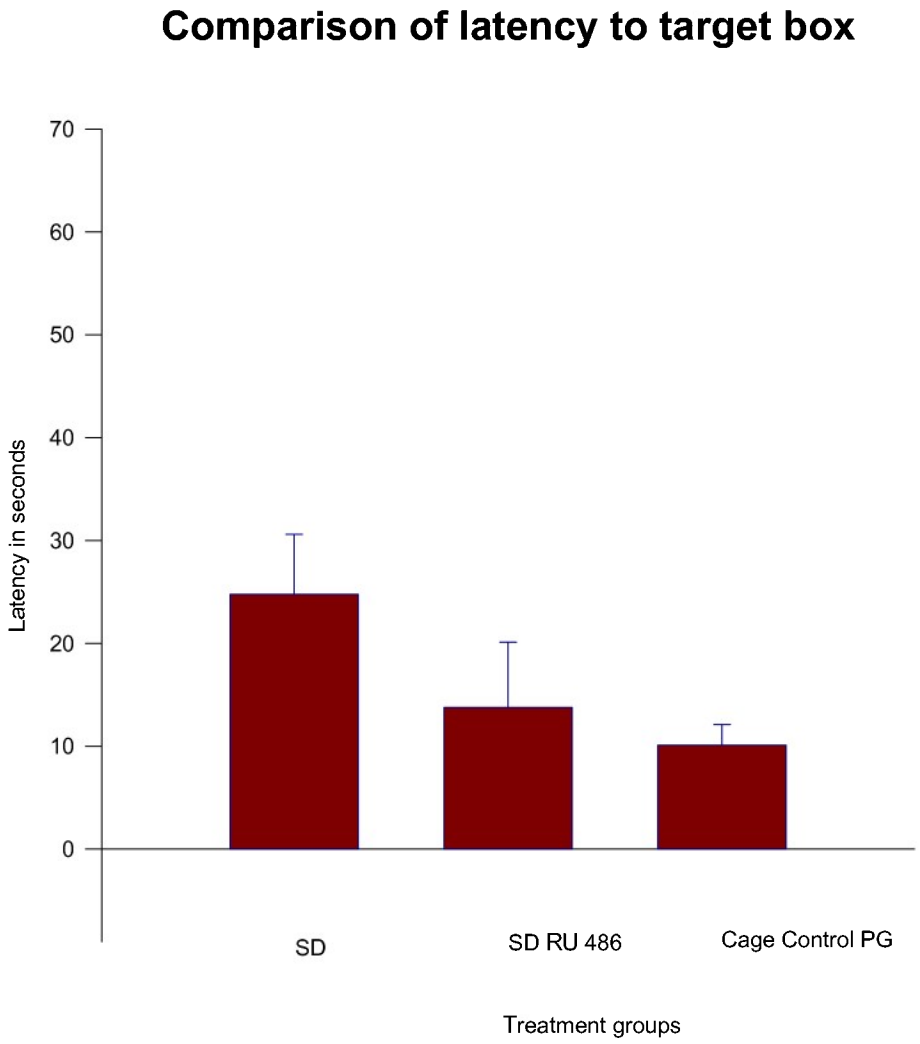


Figure 12. Comparison of latency to target box in the Barnes maze. This a comparison of the first trail of four trials in the Barnes maze after treatment with either sleep disruption, no injection (SD), sleep disruption and RU 486 3 hours prior to the trial, (SD RU 486) or cage control injected with propylene glycol 3 hours prior to the trial. The SD group *n=9,* SD RU 486 *n=9,* and Cage control PG *n=11.* The latency of the sleep disrupted group is significantly different from the SD RU 486 and Cage Control PG groups. The error bars are the standard error of the mean.

Discussion:

The hypothesis that removal of adrenal glands, i.e. corticosterone, would improve performance in sleep disrupted rats was not supported by this study. The acquisition of the radial arm maze task was not significantly different between adrenalecomtized and sham adrenalectomized animals. There was a trend for adrenalectomized animals to have more trials before reaching criterion. The similar rates of acquisition are consistent with a study comparing adrenalcetomized and intact animals in the acquisition of the Morris water maze task. (Ruskin, et al., 2006). Previously, cage control animals who had reached criterion in the radial arm maze performed with an average error rate of 0.5. However, in this study the cage controls greater variability, which may be attributed to the use of sham adrenalectomized animals as cage controls in this study. The use of a modified flowerpot protocol which involved not a deep pool of water but a cage of water 1 inch deep may also have created a confound. Because the ADX animals appeared too weak to complete 12 hours of sleep disruption in a 12 inch deep pool of water, a cage with 1 inch deep water was substituted. The difference in sleep disruption treatment makes interpretation more challenging. One interpretation is that removal of adrenal glands induced multiple changes in the hypothalamus pituitary axis, so that the single effect of removing corticosterone is one of many effects causing an outcome. Adrenalectomized animals took on average longer to navigate the maze. Although this difference in duration was not significant, it is indicative of an observed behavioral difference in mobility. In addition, the animals adrenalectomized by Harlan, appeared too ill to groom properly and up to 80% were dead within two weeks of arrival at the vivarium at UTSA. In addition, there may be compensatory mechanisms for the loss of corticosterone that help to maintain function. Another possible confound in the radial arm maze data for this study is the use of an alternative flowerpot method. Previous data collected in this lab on animals sleep disrupted for twelve hours on a flowerpot in an eleven inch deep pool of water. In this study, because of the comprised state of the adrenalectomized animals, an alternative flowerpot method was employed. The flowerpot was the same 2.3 inches in diameter, but inverted in a cage with an inch of water. The animals are sleep disrupted, however walking around in an inch of water is not as stressful as being restricted to a small platform or swimming once the animal falls off the small platform, presumably during REM sleep. Therefore, perhaps the fatigue effects were less, and therefore the effect of sleep disruption, while not confounded with as much fatigue, was more subtle and not as significant on the behavioral task of the radial arm maze. The most probable interpretation is that the increased error rate after sleep disruption is not due to the effects of elevated corticosterone, but to some other mechanism which interferes with memory or learning associated with hippocampal function. Sleep disruption during training impairs acquisition (Smith and Rose, 1996, Graves, et al,. 2003). So the effects of sleep disruption on performance of a learned task may not be associated with elevated corticosterone.

The most significant finding of this study results from using the Barnes maze to assess the impairments of sleep disruption on performance of a learned spatial task. The use of a glucocorticoid receptor antagonist, RU 486, greatly increased the efficiency of the sleep disrupted animals in finding the target box which made the latency to the target box significantly different from sleep disrupted animals and not significantly different from cage controls. The addition of the anatomical data suggests sleep disruption increases spines, which may be related to performance impairments. One study has shown that RU 486 will block the spine proliferation in the hippocampus associated with sleep disruption, which could be a mechanism for amelioration the impairments of sleep disruption.

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